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(54) Title: ANTI-CANCER PYRROLOBENZODIAZEPINE DERIVATIVES
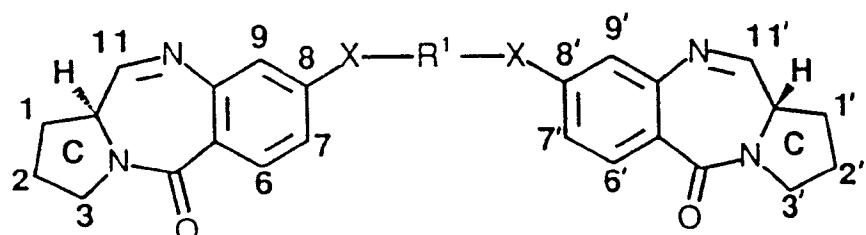
(I)

(57) Abstract

There are disclosed, as novel compounds, pyrrolo [2,1-c][1,4] benzodiazepine (PBD) dimers of formula (I) where the PBD tricyclic structures may contain additional substituents in one or more of the 1, 2, 3, 6, 7, 9 and 11-positions and the C rings may optionally contain at least one further hetero ring atom, X is -O-, -S- or -NH-, and R^1 is an alkylene chain containing from 3 to 12 carbon atoms, which chain may, if desired, be interrupted by one or more hetero-atoms and which chain may, if desired, contain one or more carbon-carbon double or triple bonds. These compounds are cytotoxic and have shown *in vitro* activity against a range of carcinoma cell lines.

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Anti-cancer pyrrolobenzodiazepine derivatives

This invention is concerned with certain novel pyrrolobenzodiazepine derivatives, with a method for their preparation and with pharmaceutical compositions containing them.

A group of pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) are known antitumour antibiotics. These compounds are produced by various Streptomyces species and well-known members of this group include anthramycin, tomaymycin, DC-81, neothramycin and sibiromycin:
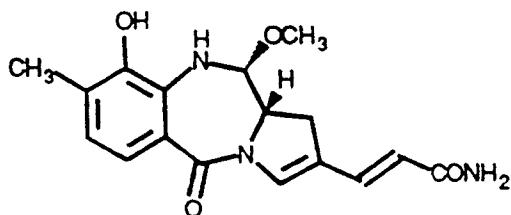
ANTHRAMYCIN
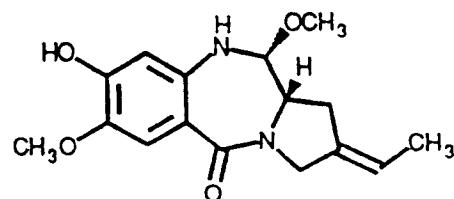
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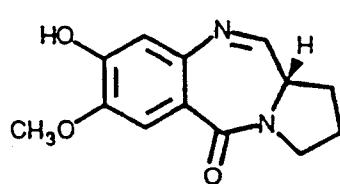
DC-81
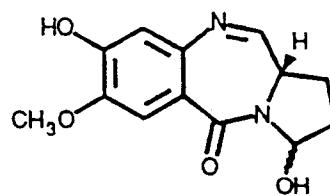
NEOTHRAMYCIN
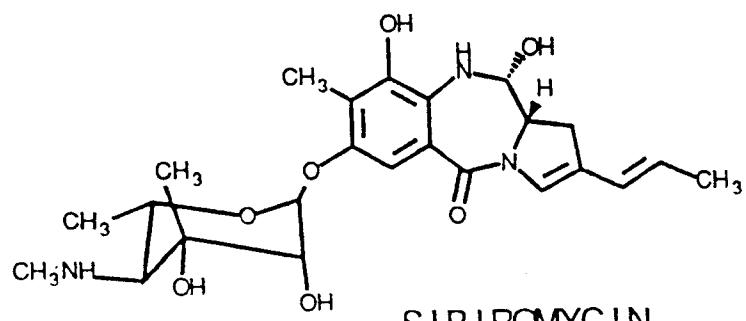
SIBIROMYCIN

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see Remers, W.A.: Pyrrolo[1,4]benzodiazepines, The
Chemistry of Antitumour Antibiotics, Vol.2, pp.28-92,
Wiley-Interscience (1988). Their antitumour and
antibacterial activity are thought to be a direct
consequence of DNA-binding which may interfere with
processes such as DNA replication or translation.

J.D. Farmer, Jr. et al, Tetrahedron Letters,
Vol 29, No. 40, pp 5105-5108, 1988 have described the
synthesis of a dimeric anthramycin analogue and its use
to effect DNA-crosslinking. The Farmer et al paper
discloses three dimers, that is compounds of the
formula:
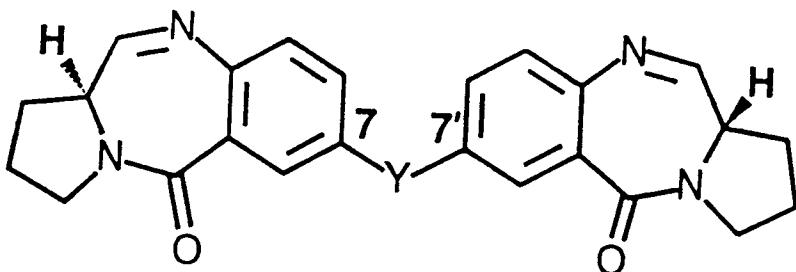
in which Y is, respectively, $-S(CH_2)_6S-$, $-O(CH_2)_2-$
$N(CH_3)(CH_2)_2O-$ and $-HN(CH_2)_3 N(CH_3)(CH_2)_3NH-$; it
appears that the third of these compounds was not
actually obtained by the synthesis described. The DNA
cross-linking ability of the second compound, with the
N-interrupted diether linkage, was examined. In order
to obtain cross-linking, the dimer was heated with DNA
at 65°C for 1 hour. An alkaline agarose gel assay
(under conditions which protected the DNA) of the dimer-
cross-linked DNA against uncross-linked DNA and
psoralen-cross-linked DNA, showed that the DNA had been
cross-linked by the dimer.

This cross-linking could be reversed, that is
the dimer could be released from the DNA, by incubating
the cross-linked DNA at a pH of 10 for 12 hours at
25°C.

It will be noted that the Farmer et al dimers

are linked through the 7-positions of the anthramycin
tricyclic structures.

We have now found that PBD dimers having a
linking group attached to the 8-positions of the PBD
structures are capable of cross-linking double-stranded
DNA significantly more readily than the dimers described
in the Farmer et al paper. Our novel dimers have
isohelicity with DNA and are capable of fitting snugly
into the minor groove of the latter.

According to one aspect of the present
invention, there are provided, as novel compounds,
pyrrolo[2,1-c][1,4]benzodiazepine (PBD) dimers of the
formula;
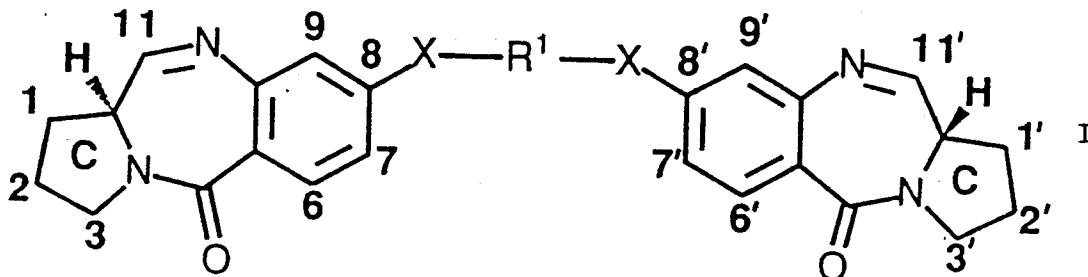
where the PBD tricyclic structures may contain
additional substituents in one or more of the 1, 2, 3,
6, 7, 9, and 11-positions and the C rings may optionally
contain at least one further hetero ring atom, X is -O-,
-S- or -NH-, and R^1 is an alkylene (i.e. polymethylene)
chain containing from 3 to 12 carbon atoms, which chain
may, if desired, be interrupted by one or more hetero-
atoms, and which chain may, if desired, contain one or
more carbon-carbon double or triple bonds.

Suitable substituents in the tricyclic rings
include, for example, hydroxyl, alkoxy, such as methoxy,
ethylidene and 2-acrylamidyl groups. Another preferred

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group of substituents have the formula:

$$-O(CH_2)_n-NH_3^+Cl^-$$
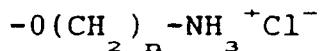
where n is an integer from 1 to 6, and are substituted
in the 2- and 2'- positions; it is generally required
that the compounds according to the invention should be
water soluble and such substituents, and also hydroxyl
substituents in the 2- and 2'- positions, have a sub-
stantial solubilising effect.

Preferred compounds according to the invention
are those in which the PBD structures have: methoxy
substituents in the 7- and 7'- positions (derived from
DC-81); methoxy substituents in the 7-, 7'-, 11- and
11'- positions and ethylidene substituents in the 2- and
2'- positions (derived from Tomaymycin); and hydroxyl
substituents in the 9- and 9'- positions, methoxy
substituents in the 11- and 11'- positions, and 2-
acrylamidyl substituents in the 2- and 2'- positions
(related to Anthramycin).

Preferred linking groups are those in which X
is -O- and R^1 is an alkylene chain containing from 3 to
6 carbon atoms or a chain of the formula $-(CH_2)_x-O-$
$(CH_2)_x-$, where x is an integer of from 2 to 6, or a
chain of the formula $-(CH_2)_n-CH=CH-(CH_2)_n-$, where n is
an integer from 1 to 5.

Whilst it has been demonstrated that a
compound according to the invention in which R^1 is
propylene, i.e. $-CH_2.CH_2.CH_2-$, effectively cross-links
the two strands of double helical DNA, that is it
effects interstrand cross-linking, it is believed that
compounds according to the invention in which the R^1
chain is of suitable length and contains at least one
rigid linkage, that is a double or triple bond, may be
capable of cross-linking two molecules of double-
stranded DNA (i.e. intermolecular cross-linking).

According to another aspect of the present

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invention, there is provided a method of preparing a PBD dimer of formula I, which comprises:

(i)   condensing a benzoic acid derivative of the formula:

$$R^4, XH, HOOC, R^3, R^2 \quad \text{II}$$
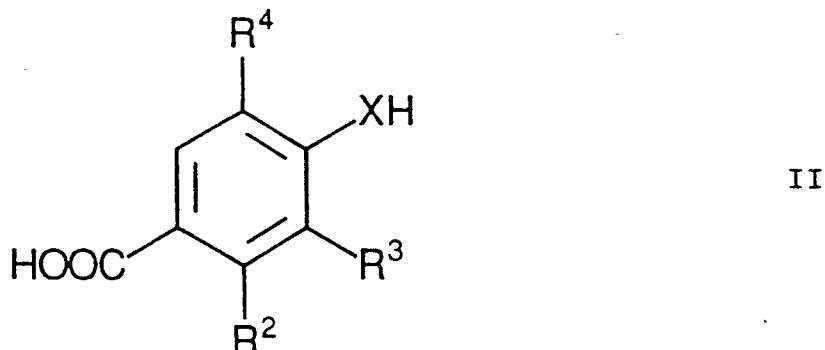
where X has the above-stated meaning (i.e. -O-, -S- or -NH-), R^2 is H or a desired substituent in the 6- or 6'-position of the PBD dimer, R^3 is H or a desired substituent in the 7- or 7'- position of the PBD dimer, and R^4 is H or a desired substituent in the 9- or 9'-position of the PBD dimer, with a dihaloalkane derivative of the formula:

$$X-R^1-X \quad \text{III}$$

where R^1 has the above-stated meaning and X is a halogen atom (preferably iodine), by adding the dihaloalkane III slowly to a reaction mixture containing the benzoic acid derivative II to form a dimer acid compound of the formula:

$$R^4, X-R^1-X, R^4, HOOC, R^3, R^2, R^3, COOH, R^2 \quad \text{IV}$$

(ii) esterifying the dimer acid of formula IV,
(iii) nitrating the diester from step (ii) to

obtain a dinitro-diester compound of the formula:

$$\text{O}_2\text{N}\!-\!\!\overset{\displaystyle R^4}{\underset{\displaystyle R^2}{\underset{\displaystyle R^5\text{OOC}}{\bigcirc}}}\!\!X\!-\!R^1\!-\!X\!\!\overset{\displaystyle R^4}{\underset{\displaystyle R^2}{\underset{\displaystyle \text{COOR}^5}{\bigcirc}}}\!\!\text{NO}_2 \qquad \text{V}$$

where R^5 is an alkyl group,

     (iv) hydrolysing the dinitro-diester compound of formula V to remove the esterifying groups and obtain the corresponding dinitro-diacid compound,

     (v) condensing the dinitro-diacid compound from step (iv) with a (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal of the formula:

$$\text{CH(SC}_2\text{H}_5)_2$$

$$\begin{array}{c}R^6\\ \text{HN}\\ R^8 \quad R^7\end{array} \qquad \text{VI}$$

where R^6 is H or a desired substituent in the 1- or 1'-position of the PBD dimer, R^7 is H or a desired substituent in the 2- or 2'- position of the PBD dimer, and R^8 is H or a desired substituent in the 3- or 3'-position of the PBD dimer, to form a nitrothioacetal derivative of the formula:

$$\text{VII}$$

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(vi)   reducing the nitro substituents of the derivative of formula VII to amino groups, and

(vii) subjecting the diamino compound from step (vi) to ring closing conditions whereby a PBD dimer of formula I is obtained.

The steps of the process according to the invention and the preferred reactants and reaction conditions for use therein, will now be described in greater detail.

(i)   The starting materials for this step, that is the benzoic acid derivatives II and the dihaloalkane derivatives III are commercially available or can be prepared by routine procedures from commercially available compounds. The dihaloalkane derivatives III include, of course, compounds in which the alkane chain is interrupted by one or more heteroatoms or by one or more double or triple bonds. Precursors of these compounds are commercially available, for example $HO-(CH_2)_x-O-(CH_2)_x-OH$ and $HO-(CH_2)_n-CH=CH-(CH_2)_n-OH$, and these can be readily converted to the corresponding dihalo compounds by standard procedures.

The condensation reaction of step (i) is preferably carried out in a two-phase reaction medium, that is a mixture of an organic solvent, such as tetrahydrofuran, in which both the reactants II and III are soluble, and an aqueous alkali, such as aqueous sodium hydroxide, a solution of the dihaloalkane III in the organic solvent being added slowly to the reaction medium which already contains the benzoic acid derivative II. We have found that by operating in this way the production of monoalkylation and elimination products (arising from the loss of HX) is minimised.

The reaction is preferably carried out at an elevated temperature, for example from 60°C to the reflux temperature of the reaction mixture.

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(ii)   The esterification may be carried out by
any of the conventional procedures. A preferred method
involves initial formation of the acid chloride using
either oxalyl chloride or thionyl chloride in an inert
solvent, such as tetrahydrofuran (THF) or benzene,
followed by treatment with an excess of methanol to form
the methyl ester. The use of other alcohols, such as
ethyl or propyl alcohol, would form the corresponding
ethyl or propyl esters.

Other methods for converting aromatic acids to
the corresponding esters include the use of dimethyl
sulphate/potassium carbonate/acetone which normally
produces high yields of methyl esters in one step. A
solution of diazomethane (CH_2N_2) gas in a solvent, such
as ether, will also convert aromatic acids directly to
the methyl ester. A conventional method of ester
synthesis involves refluxing the aromatic acid with
methanol or another appropriate alcohol in the presence
of an acid catalyst, such as sulphuric acid. A number
of more recent and more novel reagents for ester
formation are described on pages 154-156 of the well-
known book "Protective Groups in Organic Synthesis" by
Theodora W. Greene (John Wiley & Sons, 1981).

(iii)   Nitration of the product of step (ii)
may be effected by any of the standard procedures. A
preferred method comprises the use of stannic chloride
($SnCl_4$) and fuming nitric acid in methylene chloride at
-20°C.

A simple nitrating mixture of H_2SO_4/HNO_3 or
$NaNO_2$/H_2SO_4 can also be used. Other nitronium salts,
such as $NO_2^+PF_6^-$, can also be used and these are
described in a paper by Effenberger and Geke, Synthesis,
40 (1975).

(iv)   Hydrolysis of the product of step (iii)
may be carried out by any of the standard procedures. A
preferred method comprises aqueous sodium hydroxide in

THF at room temperature.

Lithium hydroxide in aqueous methanol can also be used. Other methods include the use of a nucleophile, such as lithium chloride, in an aprotic solvent (eg DMF). A mixture of $(CH_3)_3SiCl/NaI$ in CH_3CN can also effectively cleave esters. KO_2/18-crown-6 in benzene and DBN (1,5-diazabicyclo[4.3.0]non-5-ene)/xylene can also be used. These methods and others are described in detail on pages 156-159 of the book by Theodora W. Greene referred to above.

(v)   The (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal VII required for this step may be prepared in five steps from L-proline or a suitably substituted proline by the method of Langley and Thurston, _J. Org. Chem._, **52**, 91 (1987).

The condensation of the thioacetal VI with o-nitrobenzoic acid derivatives of the formula:

VIII
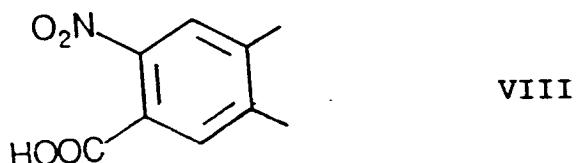
to form PBD precursors of the formula:

IX
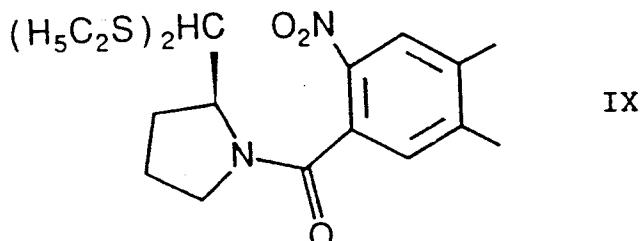
has been described in said Langley and Thurston paper and the condensation of step (v) is preferably carried out under the same conditions, that is the addition of the acid chloride to a suspension of the diethyl-thioacetal and triethylamine in water. Another method involves the use of acetonitrile as solvent in which case the water can be omitted.

(vi)   The reduction of the nitro groups of

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the product of step (v) to amino groups may be carried
out by any of the standard procedures. A preferred
method uses stannous chloride dihydrate ($SnCl_2.2H_2O$) in
methanol.

Other chemical reducing agents which can be
used include, for example, iron/acetic acid/methanol,
sodium dithionite ($Na_2S_2O_3$) in methanol, and iron
carbonyl ($Fe_3(CO)_{12}$). Sodium borohydride combinations,
such as $NaBH_4$/i-PrOH or $NaBH_4$/Pd-C, can also be used.

(vii) The final cyclization step is similar
to the cyclization step for making PBDs described by ·
Langley and Thurston, J. Org. Chem., 52, 91-97 (1987)
and is preferably carried out under the same conditions,
that is in the presence of mercuric chloride ($HgCl_2$)
and calcium carbonate ($CaCO_3$) in a mixture of
acetonitrile and water (preferably 4:1 by volume). We
have found that the reaction is complete in about 2.5
hours, as opposed to the 24 hours reported in the paper
just referred to.

We have also found that it is desirable to use
a different working up procedure from that described in
the Langley and Thurston paper. On conclusion of the
reaction, the solvents are removed by evaporation and
the residue is filtered and/or directly loaded on to a
silica gel column which is first eluted with ethyl
acetate to remove mercuric salts. Further gradient
elution with 3%-5% methanol in chloroform (HPLC grade)
allows the product to be eluted in very high yields (80%
or more compared with 15% or less using the work-up
procedure of the paper).

In a modification of the final cyclization
step, the diaminothioacetal compound from step (vi) is
reacted with sulphuryl chloride (SO_2Cl_2) in the
presence of wet silica gel in a suitable organic

solvent, such as dichloromethane. This causes ring
closure with the formation of an aminothioether
structure in the 10,11- positions, that is
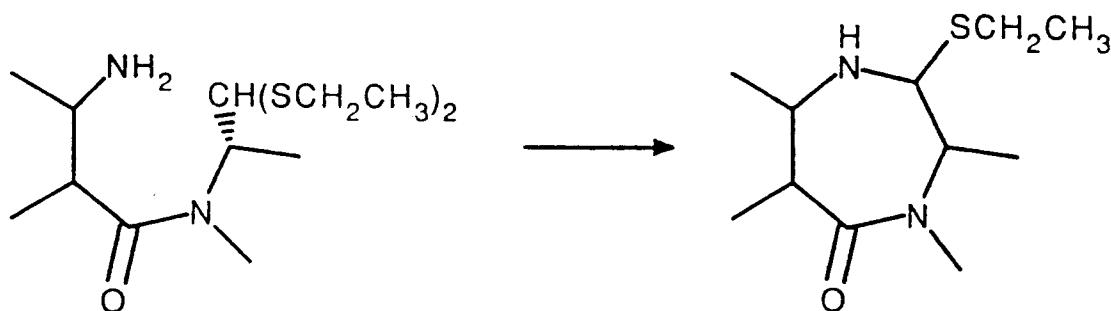
These 11- and 11'- thioether compounds are preferred
compounds according to the invention and may be isolated
and purified as such.

Alternatively they may be further treated to
obtain the imine structure, -NH=CH-, in the 10, 11-
positions by reaction with mercuric chloride or silver
nitrate in a suitable solvent, for example aqueous
methanol.

Although the modified process requires two
steps in order to obtain the imine-type compounds of
formula I from the diaminothioacetal compounds obtained
in step (vi), as against the single step of the
cyclization procedure first described, the two step
process can give a better overall yield than the one
step process and requires a less complex isolation
procedure for the desired product.

In formula I, the compounds according to the
invention are shown in the imine form at the N10-C11-
and N10'-C11'- positions. In the presence of water, the
imine form is converted to the carbinolamine form, that

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is

$$
\begin{array}{c}
OH \\
10 \quad 11 \\
- NH - C -
\end{array}
$$
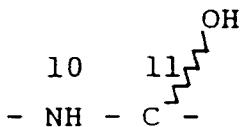
and in the presence of alcohols R^5OH, such as methanol,
ethanol and propanol, or the corresponding thiols R^5SH,
the imine is converted into the analogous ether or
thioether forms, that is

$$
\begin{array}{c}
OR^5 \\
- NH - C -
\end{array}
\qquad or \qquad
\begin{array}{c}
SR^5 \\
-NH - C -
\end{array}
$$
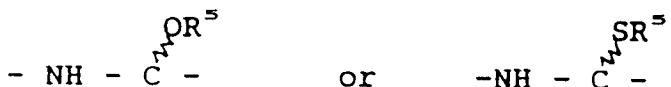
As already indicated, compounds containing such sub-
stituents in the 11- and 11'- positions (with the
nitrogen in the 10- and 10'- positions in the amine
form) are a preferred embodiment of the invention as
they are generally more stable than the corresponding
N10-C11 imine compounds from which they are derived and
since they are convertible to such imine compounds, they
are believed to constitute useful pro-drugs.

The imine form is also readily attacked by
other nucleophilic reagents, such as ethylamine and
thiophenol, or water-soluble sulphur-containing
nucleophiles, such as cysteine or glutathione, to form
substituents in the 11- position. Such nucleophiles
containing polar functional groups can enhance the
overall water-solubility of the PBD dimers. Under the
appropriate conditions, all of the C11- substituents can
be eliminated in order to reform the N10-C11 imine
species. To prepare PBD dimers according to the
invention in which the C rings contain an oxygen ring
atom requires modification of the process described
above. Steps (i) to (iv) of the above process are used
and the modified process then comprises:

(viii) condensing the dinitro-diacid compound

obtained from step (iv), or the diacid chloride
obtainable from said dinitro-diacid, with an imino
alcohol of the formula:

X
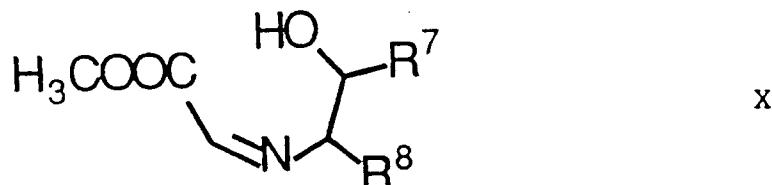
where R^7 and R^8 have the above-stated meanings, to form
a di-N-(2-nitrobenzoyl)oxazolidine ester of the
formula:

XI
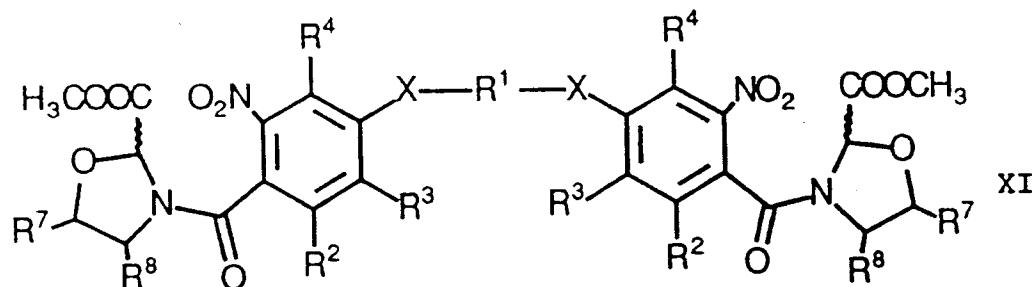
    (ix) reducing the ester groups of the
nitrobenzoyl-oxazolidine ester of formula XI to aldehyde
groups (-CHO), and
    (x) reductively cyclizing the dialdehyde from
step (ix) to form a PBD dimer of formula I (in the
10,11-carbinolamine form).
    The imino alcohol X is conveniently obtained
by condensing methyl glyoxalate with ethanolamine or an
appropriately substituted ethanolamine. This
condensation may be carried out, for example, in the
presence of methylene chloride and molecular sieves at
room temperature.
    The condensation of step (viii) may be carried
out, for example, in the presence of methylene chloride
and pyridine with cooling to 0°C.

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The reduction of the ester groups to aldehyde groups in step (ix) may be carried out using any of the reagents and conditions conventionally used for this purpose. A preferred procedure is to use di-isobutyl-aluminium hydride as the reducing agent in a toluene reaction medium at a substantially reduced temperature, for example -78°C.

The final reductive cyclization, step (x), may be carried out under mild conditions, for example by hydrogenation in the presence of Pd-C in methanol at room temperature or with stannous chloride ($SnCl_2.2H_2O$) in methanol at reflux temperature.

A particularly preferred compound according to the invention is that in which, referring to formula I, there are methoxy substituents in the 7- and 7'-positions, X is O and R^1 is propylene; this compound is referred to hereinafter as DSB-120. Its preparation is described in the Example below.

The compound DSB-120 was characterised by ^{1}H-NMR, ^{13}C-NMR, infra red, and mass spectrometry. DSB-120 is a stable, pale yellow viscous oil, highly soluble in chloroform, methanol, and ethanol and sparingly soluble in water. It is isolated in the N10- C11 imine form after purification by column chromatography; treatment of the compound in this form with methanol forms the bis(C11S-methyl ether) which is characteristic of the PBD ring system.

An _in vitro_ DNA cross-linking assay by the method described by Hartley et al, _Analyt. Biochem._, _19_, 131-134 (1990) has established that DSB-120 completely cross-links pBR322 DNA at a concentration of 1.0 μM after incubation for one hour at 37°C; a significant level of cross-linking can still be observed with concentrations of DSB-120 as low as 0.001 μM (under the same incubation conditions). The DNA cross-linking efficiency of DSB-120 is also supported by thermal

denaturation data. Treatment of calf thymus DNA with
DSB-120 at room temperature causes a 10°C increase in
helix melting temperature, compared to an untreated
control.

The PBD-dimer described by Farmer et al
apparently required incubation at 65°C for one hour at a
concentration of 250 uM to effect cross-linking of DNA,
that is to say a substantially higher concentration of
the PBD-dimer and more vigorous conditions (65°C as
against 37°C) than are required for DSB-120.

The adduct formed between DNA and DSB-120 is
also substantially more stable than that formed by
Farmer et al's PBD-dimer. Thus Farmer et al reported
electrophoretic evidence showing that their dimer adduct
was dissociated, that is the dimer was released from the
DNA, after treatment at pH10 for 12 hours at 25°C.

pBR322 DNA was cross-linked with DSB-120 at a
concentration of 0.01μM and excess DSB-120 was then
removed by precipitating and isolating the cross-linked
DNA. The latter was then incubated at 37°C at pH values
of 4.0, 7.0, 8.0, 9.0 and 10.0. A cross-linking assay
by the Hartley et al procedure referred to above showed
that after 18 hours incubation at each of these pH's, no
reversal of cross-linking had occurred. In fact, at the
lowest pH (4.0), the DNA control started to show signs
of degradation, whereas the cross-linked DNA appeared to
remain static. The stability of the cross-linked DNA
after 18 hours incubation at pH 10 is directly contrary
to the dissociation of the Farmer et al adduct after
incubation at pH 10 for 12 hours at 25°C.

Preliminary NMR data suggest that DSB-120
cross-links double-stranded DNA by binding across two
guanine residues on opposite strands with two AT base-
pairs in between.

The IC_{50} (inhibitory concentration, 50%) of
DSB-120 has been evaluated _in vitro_ in L1210, PC6 and

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CH1 cell lines using a standard screening procedure.
The table below indicates that with one exception
(mitoxantrone/CH1), DSB-120 is significantly more
cytotoxic across all cell lines than a number of known
antitumour agents. It is also noteworthy that the IC_{50}
values for DSB-120 in all cell lines are significantly
lower than those for the parent PBD (DC-81) from which
it is derived.

Compound	IC_{50} μM		
	L1210	ADJ/PC6	CH1
DSB-120	0.01	0.0005	0.003
DC-81	0.38	0.33	0.1
Cisplatin	0.5	0.02	0.1
Adriamycin	0.04	0.04	0.002
Mitoxantrone	N/A	N/A	0.0007
Melphalan	3.0	0.02	2.0
Trimelamol	20.0	5.0	15.0

According to a further aspect of the present
invention, there is provided a pharmaceutical
composition comprising a PBD dimer of formula I above
and an inert, physiologically compatible carrier.

The pharmaceutical composition may take the
form of an injectable formulation, that is a solution in
saline or saline containing a small proportion, say 10%

by weight, of ethanol or another organic solvent; or a formulation for oral administration, such as a tablet, capsule, solution or syrup; or a composition for topical applicaton, such as a gel, cream or ointment.

In addition to their physiological activity, the compounds according to the invention are useful experimental reagents for molecular biologists since they can cross-link DNA in a substantially non-reversible manner at a concentration of as low as 0.001 μM. These compounds fluoresce upon binding to DNA and may be used to stain the nuclei of cells, thus being useful in histological-type studies.

In order that the invention may be more fully understood the following examples are given by way of illustration only.

Example 1

Preparation of 1,1'- Propane-1,3-diyldioxybis(11a S)7-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[1,2-c] [1,4]benzodiazepin-5-one (DSB-120).

Reference will be made in the following description to the following reaction scheme

3: R_1 = H, R_2 = COOH
4: R_1 = H, R_2 = COOCH$_3$
5: R_1 = NO$_2$, R_2 = COOCH$_3$
6: R_1 = NO$_2$, R_2 = COOH

8: R = NO$_2$
9: R = NH$_2$
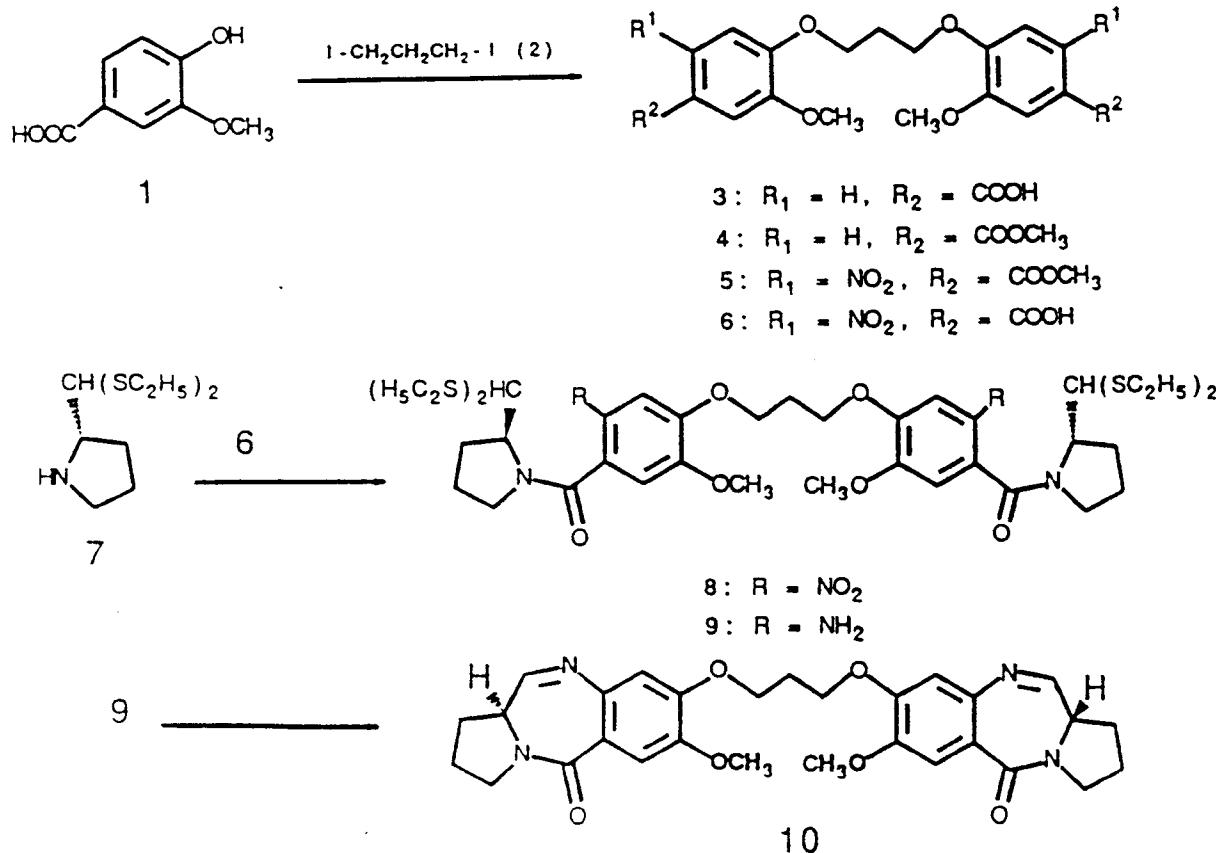
- 18-

(i)   1',3'-Bis(4-carboxy-2-methoxyphenoxy)propane (3)

Vanillic acid (1), (10g, 59.5mmol) was
dissolved in tetrahydrofuran (THF) (100ml) and aqueous
NaOH (0.5M, 225ml). A solution of 1,3-diiodopropane (2)
(8.8g, 29.7mmol) in THF (50ml) was added dropwise with
vigorous stirring at 65°C over 4 hours and then refluxed
for 48 hours. After cooling, the mixture was washed
with hexane (3 x 100ml) and the THF removed by
evaporation in vacuo. The aqueous residue was acidified
to pH 1 with HCl (36%) and the resultant precipitate was
filtered and recrystallised from acetic acid to afford
(3) as an off-white amorphous solid; yield: 7.4g (66%),
m.p. 238-240°C. MS (EI): m/z (relative intensity) =
376 (M^+, 28), 360 (3), 249 (2), 209 (45), 165 (29), 153
(16), 151 (19), 137 (19), 121 (7), 78 (15), 44 (100). IR
(KBr): $\mathcal{V}$ = 3600-2000, 1680 (C=O), 1600 (C=C), 1515,
1465, 1430, 1345, 1310, 1270, 1225, 1180, 1140, 1115,
1030, 990, 970, 950, 925, 875, 850, 825, 765, 725, 645
cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 2.23 (t, 2H,J=6Hz), 3.80 (s,
6H), 4.20 (t, 4H, J=6Hz), 7.09 (d, 2H$_{arom}$, J=8.4Hz),
7.45 (d, 2H$_{arom}$, J=1.8Hz) 7.54 (d, 2H$_{arom}$, J=8.4Hz,
1.8Hz fine coupling), 12.76 (bs, 2H$_{acid}$). ^{13}C NMR
(CDCl$_3$): δ=28.4, 55.4, 64.8, 111.9, 112.0, 122.9,
123.0, 148.3, 151.6, 167.0.

(ii) 1',3'-Bis(2-methoxy-4-methoxycarbonylphenoxy)
     propane (4)

Oxalyl chloride (3.1g, 24.4mmol, 2.4 equiv.)
was added to a stirred suspension of (3) (3.76g, 10mmol)
and dimethyl formamide (DMF) (2 drops) in dry THF
(25ml), and the stirring was continued for 6 hours. The
THF was removed by evaporation in vacuo and the
resultant solid quenched with dry methanol (60ml).
After the addition was complete, the mixture was stirred
for a further 2 hours followed by removal of the
methanol. The resultant residue was triturated with

water, and (4) was collected by filtration as a brown amorphous solid; yield: 3.8g (94%). m.p. 153-155°C. $C_{21}H_{24}O_8$ calc. 404.1472, found 404.1457: MS (EI):m/z (relatively intensity) = 404 (M^+, 92), 373 (19), 256 (3), 223 (100), 195 (49), 191 (58), 171 (18), 164 (39), 151 (51), 135 (12), 119 (20), 107 (11), 85 (61), 83 (90), 59 (23), 43 (24). IR (KBr): ν = 2940, 1710 (C=O), 1600 (C=C), 1510, 1460, 1435, 1410, 1340, 1290, 1270, 1215, 1180, 1130, 1100, 1060, 1025, 975, 870, 760, 720 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.39-2.43 (m, 2H), 3.89 (s, 6H), 3.90 (s, 6H), 4.30 (t, 4H, J=6.1Hz), 6.93 (d, 2H, J=8.5Hz), 7.54 (d, 2H, J=2.0Hz), 7.64 (dd, 2H, J=8.5Hz, fine coupling = 2.0Hz). ^{13}C NMR ($CDCl_3$): δ = 28.9. 52.0, 56.0, 65.4, 111.7, 112.3, 122.8, 123.5, 148.9, 152.2, 166.9.

(iii) 1',3'Bis(2-methoxy-4-methoxycarbonyl-5-nitro-
       phenoxy)propane (5)

A freshly prepared mixture of $SnCl_4$ (4.6g, 17.7mmol) and fuming nitric acid (1.42g, 22.5mmol) in CH_2Cl_2 (5ml) was added dropwise over 5 minutes with stirring to a solution of the methyl ester (4; 2.85g, 7.1mmol) in CH_2Cl_2 (60ml) at -25°C (dry ice/CCl_4). The mixture was maintained at the same temperature for a further 10 minutes, quenched with water (150ml), and then allowed to return to room temperature. The organic layer was separated and the aqueous phase extracted with ethyl acetate (2 x 100ml). The combined organic phase was dried (Na_2SO_4) and evaporated _in vacuo_ to afford the nitro dimer methyl ester (5) as a brown gum (3.0g) which was recrystallised from ethyl acetate/hexane to afford yellow prisms; 2.97g (85%); m.p. 165-168°C. $C_{21}H_{22}N_2O_{12}$ Calc. 494.1173, found 494.1170. MS (EI):m/z (relative intensity) = 494 (M^+, 94), 463 (14), 449 (8), 404 (3), 285 (3), 268 (28), 267 (29), 240 (32), 236 (68), 222 (94), 207 (6), 194 (45), 164 (19), 151 (15),

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122 (14), 109 (9), 86 (47), 84 (73), 75 (12), 59 (29), 49 (100), 41 (73). IR (KBr): $\mathcal{V}$ = 2940, 1710 (C=O), 1600 (C=C), 1575, 1520 (NO$_2$), 1420, 1355 (NO$_2$), 1280, 1250, 1210, 1175, 1140, 1045, 990, 960, 870, 830, 750 cm^{-1}. ^{1}H NMR (CDCl$_3$); δ = 2.43 (m, 2H), 3.90 (s, 6H), 3.95 (s, 6H), 4.32 (t, 4H, J=6.0Hz), 7.06 (s, 2H), 7.49 (s, 2H). ^{13}C NMR (CDCl$_3$): δ = 28.6, 53.3, 56.6, 65.6, 108.1, 110.9, 121.9, 149.5, 152.8, 167.0.

(iv) 1',3'-Bis(4-carboxy-2-methoxy-5-nitro-
phenoxy)propane (6)

     To a solution of (5) (0.7g, 1.4mmol) in THF (20ml) was added aq. NaOH (1M, 10ml) and the reaction mixture stirred at room temperature for 6 hours until TLC (EtOAc/hexane, 1:1) indicated that reaction was complete. The THF is removed by evaporation in vacuo, and the resultant residue acidified to pH 1 with HCl (35%). The precipitate is collected by filtration and dried to afford (6) as a yellow solid; yield: 0.62g (94%). m.p. 243-246°C. MS (EI): m/z (relative intensity) = 467 (MH$^{+\cdot}$,1), 450 (1), 436 (3), 423 (8), 378 (4), 268 (1), 255 (4), 236 (4), 210 (7), 194 (2), 182 (7), 164 (14), 153 (2), 123 (3), 91 (6), 77 (3), 55 (5), 44 (100). IR (KBr): $\mathcal{V}$ = 3620-2280, 1700 (C=O), 1595 (C=C), 1570, 1515 (NO$_2$), 1460, 1415, 1350 (NO$_2$) 1270, 1210, 1180, 1135, 1045, 930, 880, 810, 750, 730, 645cm^{-1}. ^{1}H-NMR(DMSO-d$_6$): δ = 2.25 (t, 2H, J=5.9Hz), 3.90 (s, 6H), 4.27 (t, 4H, J=5.9Hz), 7.29 (s, 2H$_{aromatic}$), 7.62 (s, 2H$_{aromatic}$), 13.6 (bs, 2H$_{acidic}$). ^{13}C-NMR (DMSO-d$_6$):δ = 28.0, 56.3, 65.7, 108.0, 111.2, 121.1, 141.3, 149.1, 151.7, 165.9.

(v) 1,1'-Propane-1,3-diyldioxybis(2-nitro-5-methoxy-1,4-
phenylene)bis(carbonyl)bis[pyrrolidine-2-carbalde-
hyde di(ethylthio)acetal] (8)

     Dimethyl formamide (2 drops) was added to a

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stirred suspension of (6) (0.5g, 1.07mmol) and oxalyl
chloride (0.34g, 2.69mmol, 2.5 equiv.) in dry THF (15ml)
and the stirring was continued for 4 hours. The THF was
removed by evaporation in vacuo, and the resultant
yellow solid was dissolved in dry THF (10ml) and added
dropwise over a period of 25 min to a vigorously stirred
suspension of (2S)-pyrrolidine-2-carbaldehyde diethyl
thioacetal (0.55g, 2.68mmol, 2.5 equiv; prepared in five
steps from (S)-proline by the method of Langley and
Thurston, J. Org. Chem., 52, 91 (1987)), triethylamine
(0.46g. 4.5mmol, 4.2 equiv.) and ice/water (0.6ml)
cooled in an ice bath. After addition was complete, the
mixture was warmed to room temperature and stirred for a
further 1.5 hours. After removal of the THF by
evaporation in vacuo, the residue was diluted with water
(2 x 50ml) and extracted with ethyl acetate (3 x 25ml).
The combined organic phase was washed with sat. $NaHCO_3$
solution (2 X 25ml), dilute HCl (2M, 2 X 25ml) and brine
(3 X 25ml), dried (anhyd. $MgSO_4$) and the solvent then
removed by evaporation in vacuo to afford a dark red oil
which was purified by flash chromatography (ethyl
acetate/hexane, 1:1; TLC: ethyl acetate/hexane, 3:2) to
afford the diamide (8) as a pale yellow oil. Yield:
0.53g (58%). IR (neat):ν2930, 1625 (C=O), 1570 (C=C),
1510 (NO_2), 1445, 1420, 1370, 1330, (NO_2), 1270, 1215,
1050, $750cm^{-1}$. 1H-NMR ($CDCl_3$) δ = 1.25-1.38 (m,14H),
1.76-2.47 (m, 8H), 2.67-2.87 (m,8H), 3.21-3.30 (m, 4H),
3.95   (s, 6H) 4.30-4.35 (m, 4H), 4.67-4.74 (m, 2H),
4.87 (d, 2H, J=3.9Hz), 6.83 (s,2H$_{aromatic}$),7.72 (s,
2H$_{aromatic}$). ^{13}CNMR δ = ($CDCl_3$) 15.0, 24.6, 26.3, 26.6,
27.2, 50.2, 52.8, 56.5, 61.1, 65.6, 108.4, 109.3, 128.5,
137.2, 148.2, 154.5, 166.5.

(vi) 1,1'-Propane-1,3-diyldioxybis(2-amino-5-methoxy-1,
      4-phenylene)bis(carbonyl)bis[pyrrolidine-2-
      carbaldehyde-di(ethylthio)acetal] (9)

        A solution of the dinitro thioacetal (8)
(0.5g, 0.59mmol) and $SnCl_2.2H_2O$ (1.37g, 6.0mmol) in
methanol (15ml) was refluxed for 40 min until TLC (ethyl
acetate/hexane, 4:1) indicated that the reaction was
complete. The solvent was removed by evaporation in
vacuo and the residue cooled to $0^{\circ}C$ and neutralised with
sat. $NaHCO_3$. The resulting solid was then triturated
with ethyl acetate (2x50ml) and the mixture was stirred
at room temperature for 1 hour. The suspension was
filtered through a short bed of celite, which was rinsed
with more ethyl acetate (2x50ml). The combined filtrate
was separated and the organic phase was evaporated in
vacuo to afford the diamino thioacetal (9) as a light
yellow foam. Further purification by flash
chromatography (ethyl acetate, 100%) afforded a yellow
oil contaminated with a small amount of solid stannic
salts, which was used directly for the next step.
Yield: 0.3g (65%). IR (neat) $\mathcal{V}$ = 3425 and 3315 (NH_2),
2930, 1620, 1590, 1505, 1460, 1455, 1400, 1260, 1165,
1030, 750 cm^{-1}.

(vii) 8,8'-[1,3-Propanediylbis(oxy)]bis[7-methoxy-
       1,2,3,11a(S)-tetrahydro-5H-pyrrolo[2,1-c]
       [1,4]benzodiazepin-5-one] (10,DSB-120)

        A solution of the diamino thioacetal (9)
(0.3g, 0.39mmol), $HgCl_2$ (0.52g, 1.92mmol, 5 equiv.), and
$CaCO_3$ (0.19g, 1.92mmol, 5 equiv.) in CH_3CN/H_2O (4:1,
15ml) was slowly stirred at room temperature for 2.5
hours until TLC (MeOH/$CHCl_3$, 1:4) indicated complete
loss of starting material. After removal of the
CH_3CN/H_2O by evaporation in vacuo at room temperature,
MeOH (15ml) was added to the residue and the suspension
filtered and/or directly loaded onto a chromatography
column (silica gel, 400 mesh). The column was first
eluted with ethyl acetate (HPLC grade) to remove the

HgCl$_2$ salts, and then with CHCl$_3$ (HPLC grade, 200ml).
Gradient elution with MeOH/CHCl$_3$ (3-5% MeOH) afforded
(10) as a light yellow oil. Yield 0.17g (83%). ^{1}H NMR
(CDCl$_3$); δ = 2.01- 2.17 (m,2H), 2.28-2.45 (m, 8H),
3.50-3.87 (m, 6H), 3.92 (s, 6H), 4.22-4.33 (m, 4H),
6.85 (s, 2H), 7.5 (s, 2H), 7.66 (d, 2H, J=4.4Hz). ^{13}C
NMR (CDCl$_3$):   δ = 24.2, 28.8, 29.6, 46.7, 53.7, 56.1,
65.4, 110.7, 111.6, 120.3, 140.6, 147.8, 150.6, 162.4,
164.6. MS (FAB): m/z=533 (M$^+$+1). Conversion to the
C11(S) methyl ether form was effected by dissolving in
CH$_3$OH: ^{1}H-NMR (CD$_3$OD) : δ = 2.0-2.3 (m, 10H,) 3.3 (s,
6H), 3.47-3.72 (m, 6H), 3.8 (s, 6H), 4.22-4.23 (m, 4H),
4.40 (d, 2H, J=9.0Hz), 6.61 (s, 2H), 7.15 (s, 2H), 7.90
(s,2H). A small amount of the C11(R) isomer was
evidence from signals at 4.58 (s, H11), 6.35 (s) and
7.34 (s).[α]$_D^{23}$=+330°(c=0.6,CHCl$_3$).

Example 2

DNA cross-linking efficiency was evaluated
using the method of Hartley et al (Analyt. Biochem.,
19, 131-134 (1990)). The reaction of DSB-120 with
double-stranded, end-labeled, pBR322 DNA in a
concentration range of 0.001-100μM was allowed to
proceed in a triethanolamine/EDTA (25mM:1mM, pH7.2)
buffer at 37°C. After 1h incubation, the reaction was
stopped by the addition of an equal volume of sodium
acetate/EDTA (0.6M:20mM) buffer and tRNA (100μg/ml).
The DNA was precipitated by the addition of 95% ethanol
(3 vols), and the resulting pellet, after centrifugation
and removal of the supernatant, was dried and
lyophilized. Samples were redissolved in a strand
separation buffer (10μM of 30% DMSO/1ml EDTA/0.04%
bromophenol blue/0.04% xylene cyanol), heated to 90°C
for two minutes to ensure denaturation, and then chilled
in ice water immediately prior to gel electrophoresis.

Controls consisting of undenatured samples
were dissolved directly in loading buffer (10μg of 6%
sucrose/0.04% bromophenol blue) prior to loading.

Electrophoresis was performed on 20cm

submerged agarose gels (0.8%) at 40V for 16h. The gel
and running buffer consisted of 40mM Tris, 20mM acetic
acid and 2mM EDTA (pH 8.1). Autoradiography relative
band intensities were determined by microdensitometry
using a LKB Ultrascan-XL laser densitometer.

These experiments showed that DSB-120
completely cross-links the experimental DNA at a
concentration of 1.0 µM and that significant cross-
linking is observed down to a concentration of 0.001 µM.

The stability of the adduct to low pH and high
temperature was also investigated. A sample of end-
labelled linearised pBR322 DNA previously treated with
DSB-120 was centrifuged to precipitate cross-linked DNA,
and then unreacted DSB-120 was removed in the
supernatant. The cross-linked DNA was then incubated
for 15 minutes in buffers at pH values 4.0, 7.0, 8.0,
9.0 and 10.0. Following the procedure of the cross-
linking assay described above, no reversal of cross-
linking was observed at these pH values. In addition,
heating the adducts at 90°C for two minutes which is
part of the assay protocol, also failed to reverse
cross-linking.

Examples 3-5

      Ex. 3: 8,8'-[1,4-Butanediylbis(oxy)]bis[7-
            methoxy-1,2,3,11a(S)-tetrahydro-5H-
            pyrrolo[2,1-c][1,4]benzodiazepin-5-
            one]; (AT-111)

      Ex. 4: 8,8'-[1,5-Pentanediylbis(oxy)]bis[7-
            methoxy-1,2,3,11a(S)-tetrahydro-5H-
            pyrrolo[2,1-c][1,4]benzodiazepin-5-
            one]; (AT-286)

      Ex. 5: 8,8'-[1,6-Hexanediylbis(oxy)]bis[7-
            methoxy-1,2,3,11a(S)-tetrahydro-5H-
            pyrrolo[2,1-c][1,4]benzodiazepin-5-
            one]; (DSB-131)

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The above three compounds of formula I in which X is -O-, R^1 is butylene, pentylene, and hexylene, respectively, and the 7- and 7'- positions are substituted by methoxy groups, were prepared by the method described in Example 1 with the appropriate substitutions of the diiodoalkane reagent (2). Thus in Example 3, 9.2g of 1,4-diiodobutane were used in place of 1,3-diiodopropane; in Example 4, 9.6g of 1,5-diiodopentane; and in Example 5, 10.0g of 1,6-diiodohexane.

The characteristics of these compounds and the yields of the final steps of the synthesis were as follows.

EX. 3

^{1}H-NMR (CDCl$_3$): δ = 2.02-2.17 (m), 2.29-2.34 (m,7H), 3.50-3.87 (m), 3.93 (s, 6H), 4.12-4.21 (m, 4H), 6.81 (s, 2H), 7.50 (s, 2H), 7.66 (d,2H, J=4.4Hz). ^{13}C-NMR (CDCl$_3$): δ = 24.2, 25.7, 29.6, 46.7, 53.7, 56.1, 65.4, 110.7, 111.6, 120.3, 140.6, 147.8, 150.6, 162.4, 164.6, Yield 65% from 0.52g (0.65mmol).

EX. 4

^{1}H-NMR(CDCl$_3$): δ = 1.61-1.66(m), 1.95-2.04(m), 2.28-2.30 (m), 3.57-3.84(m), 3.93(s), 3.97-4.07(m), 6.80(s,2H), 7.51 (s,2H), 7.64(d, 2H, J=11.9Hz): ^{13}C-NMR(CDCl$_3$): δ = 24.2, 22.5, 28.6, 29.7, 46.7, 53.7, 56.2, 68.5, 110.4, 111.5, 120.1, 140.6, 147.8, 150.8, 162.4, 164.7: Yield 60% from 0.53g (0.65mmol). [α]$_D^{23}$=+47.8°(c=0.4, CHCl$_3$).

### EX. 5

^{1}H-NMR(CDCl$_3$): δ = 1.54-1.56 (m,4H), 1.89-2.08 (m,8H), 2.11-2.33 (m,4H), 3.47-3.85 (m,6H), 3.89 (s,6H), 4.03-4.09 (m,4H), 6.80 (s,2H), 7.50(s,2H), 7.66(d,2H, J=4.4Hz): ^{13}C-NMR(CDCl$_3$): δ = 24.2, 25.7, 28.8, 29.6, 46.6, 53.7, 56.2, 68.8, 110.4, 111.5, 120.1, 140.6, 147.8, 150.8, 162.4, 164.7: Yield 82% from 0.53(0.65mmol). [α]$_D^{23}$=+470.5° (c=0.18,CHCl$_3$).

Example 6

8,8'-[1,3-Propanediylbis(oxy)]bis[(E,Z)-
2-ethylidene-7-methoxy-1,2,3,11a(S)-tetra-
hydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-
one]: (17)

This compound of formula I in which X is -O-,
R^1 is propylene, the 2- and 2'- positions are
substituted by ethylidene groups, and the 7- and 7'-
positions are substituted by methoxy groups, was
prepared by a method generally similar to the method of
Example 1 subject to the replacement of the reactant
(2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal
(compound 7 of Example 1) by the compound, (2S)-(E,Z)-4-
ethylidenepyrrolidine-2-carboxaldehyde diethyl
thioacetal (16), of the formula:

16
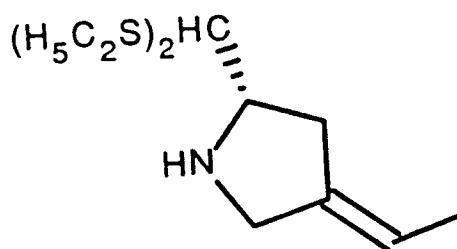
Compound 16 was prepared from (2S)-N-(benzoxy-
carbonyl)-4-oxo-pyrrolidine-2-carboxylic acid (11) by
the following reaction scheme:
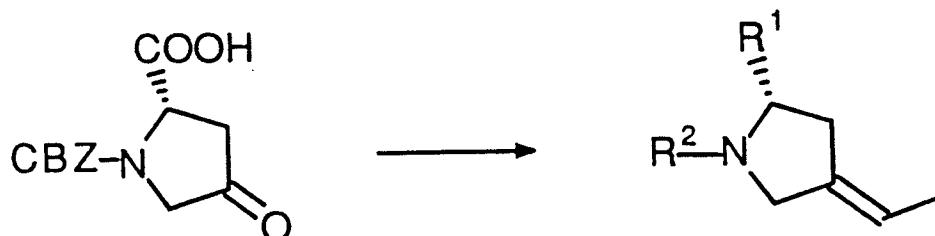
11

12: R'= COOH, R^2 = CBZ

13: R'= COOCH$_3$, R^2 = CBZ

14: R' = CHO, R^2 = CBZ

15: R' = CH(SEt$_2$)$_2$, R^2 = CBZ

16: R' = CH(SEt$_2$)$_2$, R^2 = H

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(2S)-(E,Z)-N-(Benzoxycarbonyl-4-ethylidenepyrrolidine-2-
carboxylic acid (12)
_____

Potasium t-butoxide (18.6g, 152mmol, 4.0 equiv) and tri-
ethylphosphonium bromide (28.2g, 76mmol, 2.0 equiv) were
added to a stirred solution of (2S)-N-(benzoxycarbonyl)-
4-oxo-pyrrolidine-2-carboxylic acid (11;10.0g, 38mmol)
in freshly distilled and dried (sodium) THF (300ml).
The mixture was stirred at room temperature under dry
nitrogen for 30 minutes and then refluxed for 6 hours.
The resulting mixture was filtered and the filtrate
evaporated _in vacuo_ to afford a crude brown oil.
Further purification by flash chromatography (1:1, ethyl
acetate/hexane) gave 6.79g (56%) of a mixture of E and Z
isomers as a golden yellow oil.
^{1}H-NMR (CDCl$_3$): δ=1.60-1.63 (m,3H.=CHC$\underline{H}_3$), 2.66-3.00
(m,2H,-N-CH$_2$-CO-C$\underline{H}_2$-), 3.98-4.20 (m,2H,-N-C$\underline{H}_2$-CO-CH$_2$-),
4.50-4.58 (m,1H , -C$\underline{H}$-COOH), 5.09-5.28 (m,2H, -C$\underline{H}_2$-O-
CO-), 5.41 (bs, 1H,=C$\underline{H}$-CH$_3$), 7.25-7.50 (m, 5H$_{arom}$), 7.60
(bs, 1H, -COO$\underline{H}$); ^{13}C-NMR (CDCl$_3$) [E and Z isomers]
(rotomers): δ = (14.4, 14.7),[(31.4,32.5)(35.4, 36.5)],
[(47.8,48.3)(50.8,51.1)][(58.3,58.5,58.7,59.0)],
(67.3,67.5),(118.0,118.4),(127.9,128.4,128.5),(132.8,132
.9),133.7,(136.3, 136.4), (154.5,155.6), (176.7,177.6);
IR (neat film) υ=2500-3600, 1650-1750(C-O),1585, 1500,
1420,1350,1295,1230,1120,1050,1025,950,910,750,700;MS
(ACE,isobutane): m/z(relative intensity) = 275(M$^+$,6),230
(8), 201 (4),186(26),140(70),96(15),91(100),65(22);
MS(CI,isobutane)276(MH$^+$),232

Methyl(2S)-(E,Z)-N-(benzoxycarbonyl)-4-ethylidene-
pyrrolidine-2-carboxylate(13)
_____
Oxalyl chloride (75g,0.59mol) was added dropwise over 30
minutes to a stirred solution of (2S)-(E,Z)-N-(benzoxy-
carbonyl)-4-ethylidenepyrrolidine-2-carboxylic acid (12;
80g,0.27mol)dissolved in dry toluene (400ml) at 0°C
(ice/water bath). Four drops of DMF was added and

stirring continued for 12 hours at room temperature.
Freshly distilled dry methanol (200mL) was added to the
mixture and the reaction stirred for a further 4 hours.
The solvent was evaporated in vacuo and the residual oil
dissolved in ethyl acetate (100mL), washed with
saturated aqueous NaHCO$_3$ (4 x 50mL), brine (100mL),
then dried (MgSO$_4$) and the solvent evaporated in vacuo
to afford 79g (100%) of 13 as a pale yellow oil;
^{1}H-NMR (CDCl$_3$): δ=1.59-1.63 (m,3H,=CH-C$\underline{H}$$_3$),2.57-2.68 and
2.83-3.00 (m,2H,-N-CH$_2$-CO-C$\underline{H}$$_2$-), 3.72 and 3.73 (s,3H, -
COOC$\underline{H}$$_3$), 4.07-4.12 (m,2H,-N-C$\underline{H}$$_2$-CO-CH$_2$), 4.46-4.62 (m,
1H,-C$\underline{H}$-COOH), 5.04-5.11(m,2H,-C$\underline{H}$$_2$-O-CO-), 5.40 (bs, 1H,
=C$\underline{H}$-CH$_3$), 7.27-7.38 (m,5H$_{arom}$); ^{13}C-NMR(CDCl$_3$) [E and Z
isomers] (rotomers): δ=(14.5,14.7),[(31.7,32.6)(35.7,
36.5)],[(47.8,48.4)(50.7,51.1)],52.2,53.6,[(58.5,58.7,
58.9)], 67.1, [(117.7, 117.8, 118.2)], 127.8, 127.9,
128.4, [(133.1, 133.2)(134.0,134.1)], [(136.5,136.6)],
[(154.3,154.5)(154.9,155.0)], 172.9; IR (neat film) ν =
2800-3100, 1745 (C=O), 1705 (C=O),1410,1350,1210,1180,
1115, 1025, 775, 700cm^{-1}; MS (EI): m/z (relative
intensity) =289 (M$^+$,2), 244 (8), 230 (87), 186 (20), 154
(15), 91 (100), 85 (12), 65 (35); MS (CI, isobutane);
290 (MH$^+$)

(2S)-(E,Z)-N-(Benzoxycarbonyl)-4-ethylidenepyrrolidine-
2-carboxaldehyde(14)

A solution of (i-Bu)$_2$AlH [DIBAL-H] (100mL of a
1M solution in toluene) was added dropwise over a
period of 45 minutes to a solution of the methyl ester
(13;10g, 34.6mmol) in dry toluene (150mL) maintaining
the temperature at -78°C (acetone/dry ice) under a
nitrogen atmosphere. The reaction was stirred for a
further 30 minutes and then the excess DIBAL-H carefully
decomposed by the addition of methanol (100mL) followed
by NaOH(0.2M, 100mL). The mixture was allowed to warm
to room temperature and the organic layer separated.
The aqueous layer was adjusted to pH 11 (NaOH,0.5M) and

extracted with ethyl acetate (3 x 100mL). The ethyl
acetate extracts were combined, washed with brine
(100mL), dried (MgSo$_4$) and the solvent evaporated in
vacuo (<35°C) to afford 5.83g (65%) of 14 as a pale
yellow oil. TLC: (ethyl acetate/petroleum ether 1:1)
^{1}H-NMR (CDCl$_3$): δ=1.60-1.63 (m,3H, =CH-C$\underline{H}_3$), 2.40-2.90
(m, 2H, -N-CH$_2$-C-C$\underline{H}_2$-), 4.08-4.12 (m, 2H,-N-C$\underline{H}_2$-C-CH$_2$-),
4.15-4.67 (m, 1H, -C$\underline{H}$-COOH), 5.14-5.25 (m, 2H, -C$\underline{H}_2$-O-
CO-), 5.42 (bs, 1H,=C$\underline{H}$-CH$_3$), 7.26-7.36 (m, 5H$_{arom}$), 9.48
(d,0.35H,J=6.32Hz, Z isomer, 35%, -CH-C$\underline{H}$O) and 9.56 (d,
0.65H, J=6.9Hz, E isomer, 65%, -CH-C$\underline{H}$O); ^{13}C-NMR
(CDCl$_3$) [E and Z isomers] (rotomers): δ = 14.4,
[(28.0,29.0)(32.0,33.0)], [(48.0,48.5)(51.0,51.5)],
(64.6,64.7), 67.4, [(118.4, 118.9)], 128.0, 128.2,
128.5, [(132.4, 133.4)], 136.3, [(155.0, 156.0)],
[(199.3, 199.5)]' IR (neat film)ν=2700-3700, 2900, 1720
(C=O), 1700 (C=O), 1500, 1415, 1350, 1300, 1200, 1180,
1110, 1060, 920, 770, 735, 700cm^{-1}; MS (EI) m/z
(relative intensity) =230(50,M$^+$.-CHO), 186 (37), 154
(9), 91 (100), 65 (21).

(2S)-(E,Z)-N-(Benzocarbonyl)-4-ethylidenepyrrolidine-2-
<u>carboxaldehyde diethyl thioacetal (15)</u>
Ethanethiol (7.90g. 127mmol) was added to a stirred
solution of the aldehyde (14;15g, 58mmol) in freshly
distilled dichloromethane (40mL) under a nitrogen
atmosphere. The mixture was stirred for a further 30
minutes followed by the addition of trimethylsilyl
chloride (11.65g, 107mmol). The reaction was then
stirred under nitrogen for a further 24 hours or until
TLC (ethyl acetate/hexane 1:1) indicated that the
reaction was complete. The mixture was then carefully
neutralised with saturated aqueous NaHCO$_3$ and then
extracted with water (3 x 30mL). The combined aqueous
phase was back-extracted with chloroform (3 x 20mL) and
the combined organic phase dried (MgSO$_4$) and evaporated

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in vacuo to afford 16.91g (80%) of the thioacetal (15)
as a dark brown oil.

^{1}H-NMR (CDCl$_3$) δ=1.09-1.39 (m, 6H, CH(SCH$_2$CH$_3$)$_2$), 1.56-
1.65 (m, 3H, =CH-CH$_3$), 2.40-2.60 (m,4H, CH(SCH$_2$CH$_3$)$_2$),
2.60-2.81 (m, 2H, -N-CH$_2$-C-CH$_2$), 4.05-4.20 (m,2H,-N-CH$_2$-
C-CH$_2$), 4.25-4.58 (m, 2H, CH-CH(SCH$_2$CH$_3$)$_2$ and CH-
CH(SCH$_2$CH$_3$)$_2$), 5.02-5.15 (m, 2H, -CH$_2$-O-CO), 5.41 (bs,
1H,=CH-CH$_3$), 7.12-7.41 (m, 5H$_{arom}$); IR (neat film) ν =
3200-3600, 2800-3100, 1700 (C=O), 1450, 1410, 1350,
1300, 1265, 1245, 1205, 1105, 1045, 975, 920, 750, 700,
cm^{-1}; MS (EI) m/z (relative intensity) =365 (M$^{+\cdot}$;3), 303
(12), 292 (4), 230 (43), 186 (53), 168 (6), 152 (9), 135
(16), 107 (6), 91 (100), 75 (5), 65 (14); Calculated for
C$_{19}$H$_{27}$NO$_2$S$_2$ 365.1483 found 365.1497.

(2S)-(E,Z)-4-Ethylidenepyrrolidine-2-carboxaldehyde
diethyl thioacetal (16)
A solution of trimethylsilyl iodide (5.0mL, 35.4mM, 2.4
equiv) in freshly distilled dry dichloromethane (75mL)
was added dropwise with stirring to a solution of
15;5.4g, 14.7mM, 1 equiv) and 2,3-dimethylbutene (5.0ml)
in dichloromethane (50mL) under a nitrogen atmosphere.
The mixture was stirred for 90 minutes or until reaction
was complete as indicated by TLC (ethyl acetate/hexane,
1:9). After quenching with methanol (100mL) and
evaporation of the solvent at 35°C in vacuo, the
resulting oil was dissolved in ether (150mL) and
extracted with HCl (0.5M, 3 x 200 mL). The combined
aqueous phase, after adjusting to pH8.0 with 2M NaOH,was
back-extracted with ether (4 x 150mL) and the combined
organic phase dried (MgSO$_4$) and evaporated in vacuo to
afford a crude red oil which was purified by column
chromatography (silica gel 400 grade, hexane/ethyl
acetate, gradient 9:1 to 4:1) to afford 2.10g (65%) of
the amine 16 as a dark brown oil.

^{1}H-NMR (CDCl$_3$): δ= 1.16-1.20 (m, 6H, CH(SCH$_2$CH$_3$)$_2$), 1.55

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(d, 1H, J-6.8Hz, Z-isomer, 35%, =C$\underline{H}$-CH$_3$), 1.61 (d, 2H,
J=6.8Hz, E-isomer, 65% =C$\underline{H}$-CH$_3$), 2.49-2.80 (m, 6H, -N-
C$\underline{H}_2$-C-C$\underline{H}_2$ and CH(SC$\underline{H}_2$CH$_3$)$_2$), 3.24-3.70 (m, 3H, N-C$\underline{H}_2$-C-
CH$_2$ and C$\underline{H}$-CH(SCH$_2$CH$_3$)$_2$), 3.75 (d,0.35H, J=8.2Hz, Z-
isomer, 35%, CH-C$\underline{H}$(SCH$_2$CH$_3$)$_2$) and 3.80 (d,0.65H,
J=8.1Hz, E-isomer, 65%, CH-C$\underline{H}$(SCH$_2$CH$_3$)$_2$), 5.32 (bs, 1H,
=C$\underline{H}$-CH$_3$); ^{13}C-NMR (CDCl$_3$) [E and Z isomers] (rotomers):
δ=[14.5, 14.8, 14.6], [24.2, 24.4, 25.0], [33.7, 37.5],
[47.8, 51.2], [56.5, 56.7], [61.9, 62.3], [115.0,
115.4], [139.1, 139.2]; IR (neat film) ν=2950, 2920,
1575, 1450, 1360, 1175, 1100, 1050, 975, 845, 815, 780,
745, 695, 600cm^{-1};

The process of Example 1 was then followed to
give 8,8'-[1,3-propanediylbis(oxy)]bis[(E,Z)-2-
ethylidene-7-methoxy-1,2,3,11a(S)-tetrahydro-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one], (17) as a light
yellow oil.
^{1}H-NMR(CDCl$_3$):δ=1.67 and 1.73 (s, 6H, C=CH-C$\underline{H}_3$, E and
Z), 2.28-2.45 (m, 2H), 2.95-3.05 (m, 4H), 3.83-3.88 (m,
4H), 3.92 (s, 6H), 4.20-4.30 (m, 4H), 5.58 (bs, 2H),
6.88 (s, 2H), 7.48 (s, 2H), 7.66 (d, 2H); MS (FAB): m/z
=584 (M$^{+\cdot}$), 505, 490, 479, 465, 448, 425, 415, 359, 343,
329, 311, 301 (586 (M$^{+\cdot}$+2) was also observed);
C$_{33}$H$_{36}$O$_6$N$_4$ calculated. 584.2635, found 584.2743; Yield
55% from 0.80g.

Example 7

8,8'-[Oxybis(ethane)-2,2'-diylbis(oxy)]bis[7-
methoxy-1,2,3,11a(S)-tetrahydro-5H-pyrrolo-
[2,1-c][1,4]benzodiazepin-5-one](18)

This compound of Formula 1 in which X is -O-,
R^1 is -(CH$_2$)$_2$-O-(CH$_2$)$_2$-, and the 7- and 7'- positions
are substituted by methoxy groups, was prepared by the
method of Example 1, but using the compound 1,1'-

oxybis(2-iodethane), 19, of the formula:

$$I-CH_2CH_2-O-CH_2CH_2-I \qquad 19$$

in the first step in place of diiodopropane.

Compound (19) was prepared from 1,1'-oxybis[2-chloroethane] as follows:

1.1'-Oxybis[2-iodoethane] (19)

Sodium iodide (125g, 0833mol, 7.1 eqiv) was added to a solution of 1,1'oxybis[2-chloroethane] (16.65g, 0.11mol) in dry acetone (300ml). The solution was then heated under reflux for 40 hours, cooled and the sodium salt filtered. The acetone was evaporated in vacuo. To the residue was added diethyl ether (300ml) and the organic layer washed with sat.aq. solution of sodium thiosulfate (3 x 50ml). The organic layer was dried (Na_2SO_4) and evaporated in vacuo to afford a yellow oil, yield 29.72g (95%). MS (EI): m/z (relative intensity) = 326 (M^+,42), 277 (2), 199 (26), 155 (100), 127 (11); IR (KBr): = 2960, 2880, 1465, 1435, 1420, 1360, 1310, 1270, 1190, 1165, 1110, 1035, 980, 775 cm^{-1}. ^{1}H NMR ($CDCl_3$):δ = 3.26 (t, 4H, J=6.96 Hz), 3.77 (t, 4H, J=6.96 Hz).

The procedure of Example 1 was then followed to give compound (18) above as a light yellow oil. MS (FAB): m/z (relative intensity) =563, (M^++1, 100).^{1}H-NMR ($CDCl_3$) : δ=2.05-2.17 (m, 12H), 2.28-2.36 (m, 6H), 3.49-3.89 (m, 6H), 3.92 (s, 6H), 4.01 (t, 4H), 4.18-4.30 (m, 6H), 6.83 (s, 2H$_{arom}$), 7.50 (s,2H$_{arom}$), 7.66 (d, 2H, J=4.4Hz). ^{13}C-NMR ($CDCl_3$): δ=24.16, 29.55, 46.70, 53.71, 56.12, 68.38, 69.62, 110.78, 111.62, 120.50, 140.47, 147.82, 150.59, 162.50, 164.61.

Example 8

8,8'-[1,3-Propanediylbis(oxy)]bis[2(S),11(R,S),11a(S)-7,11-dimethoxy-1,2,3,10,11,11a-hexa hydro-2-hydroxy-5H-pyrrolo[2,1-c][1,4]-benzodiazepin-5-one] (26)

This compound of formula I in which X is -O-,

R^1 is propylene, the 2- and 2'- positions are
substituted with hydroxyl groups and the 7-, 7'-, 11-,
and 11'- positions (with the 10,11- and 10', 11'-
positions in the carbinolamine form) are substituted
with methoxy groups, was prepared by a process analogous
to that of Example 1 as follows.

Methyl(2S)-N-(Benzoxycarbonyl)-(4S)-hydroxypyrrolidine-
carboxylate (20)

DMF (2 drops) was added to a suspension of the Cbz-acid
(10.6g, 40mmol) and oxalyl chloride (7.6g, 59.9mmol,
5.2ml) in anhydrous THF (40ml), and the mixture stirred
at room temperature for 5h until TLC (EtOAc/hexane, 1:1)
indicated complete reaction. The reaction mixture was
evaporated in vacuo, the residue was then quenched with
methanol (2 X 25ml) and the mixture stirred for a
further 1h. After evaporation of the solvent, the
resultant residue was dissolved in EtOAc(2 X 50ml). The
solution was washed with aq. $NaHCO_3$ (3 X 50ml), brine (2
X 25ml), and then dried ($MgSO_4$) prior to evaporation in
vacuo to afford 10.1g (90%) of pure 20 as a yellow oil.
^1H-NMR ($CDCl_3$): δ 2.01-2.12 (m, 1H), 2.24-2.39 (m, 2H),
3.54 (s, 3H), 3.56-3.66 (m, 2H), 4.46-4.54 (m, 2H),
5.02-5.21 (m, 2H), 7.26-7.36 (m, 5H). ^{13}C-NMR ($CDCl_3$,
rotamers in brackets): δ 38.4 (39.2), 52.14 (52.4), 54.6
(55.2), 57.7 (57.9), 67.3, 69.3 (70.1), 127.8, 127.9,
128.0, 128.4, 128.5, 136.2 (136.4), 154.6 (155.1), 173.1
(173.2). IR (Neat):ν 3600-3150, 2950-2800, 1745, 1680,
1420, 1350, 1200, 1160, 1120, $1080cm^{-1}$. MS (m/z) (EI,
relative intensity): 279 (64), 220 (77), 176 (79), 158
(9), 144 (22), 110 (16), 92 (57), 91 (100), 65 (36).
HRMS Calcd.for 279.1107 ($C_{11}H_{17}NO_5$), found 279.1106.
$[\alpha]_D^{20}$ = -75.8° (c=2.6,$CHCl_3$).
(2S)-N-(Benzoxycarbonyl)-4(S)-hydroxypyrrolidine-2-
carboxaldehyde (21)

The Cbz-ester (20;3.51g, 12.6mmol) was dissolved in
anhydrous toluene (80ml) and diisobutylaluminum hydride

solution (4.5g, 31.5mmol, 21ml of a 1.5M solution in
toluene) added dropwise over a period of 30min at -78°C
(liq.N$_2$/acetone) under a N$_2$ atmosphere. The mixture was
maintained at the same temperature for a further 50min
until TLC (EtOAc/hexane, 1:1) indicated complete loss of
starting material. The mixture was quenched with
methanol (20ml) followed by HCl (1M, 35 ml) and allowed
to warm to room temperature. The organic layer was
separated and the aqueous phase extracted with EtOAc (4
X 50ml). The combined organic phase was back washed
with water (2 X 125ml), brine (2 x 75ml) dried (MgSO$_4$)
and the solvent removed _in vacuo_ to afford a yellow oil,
which was purified by chromatography (EtOAc/hexane, 6:4)
to afford 1.9g (67%) of the corresponding aldehyde 21 as
a light yellow oil. ^{1}H-NMR (CDCl$_3$): δ 1.91-2.27 (m,
2H), 2.60 (bs, 1H), 3.52-3.68 (m, 2H), 4.21-4.55 (m,
2H), 5.05-5.20 (m, 2H), 7.29-7.40 (m, 5H), 9.45 and 9.55
(d, 1H, rotomers). ^{13}C-NMR (CDCl$_3$, rotomers in
brackets): δ 35.2 (36.0), 55.0 (55.7), 63.5 (63.6),
67.6, 69.1 (70.0), 127.8, 128.0, 128.1, 128.2, 128.5,
135.9 (136.1), 154.9, 199.3 (199.5). IR (Neat);ν 3600-
3150, 3030-2800, 1730, 1685, 1415, 1150, 1110, 1050,
990, 750cm^1.

(2S)-N-(Benzoxycarbonyl)-4(S)-hydroxypyrrolidine-2-
carboxaldehyde diethyl thioacetal (22)

Ethanethiol (1.1g, 17.9mmol), was added to a stirred
solution of the Cbz-aldehyde (21;1.8g, 7.15mmol) in dry
CHCl$_3$ (20ml) under a N$_2$ atmosphere. The mixture was
stirred for 40min, followed by the addition of
trimethylsilyl chloride (1.93g, 17.8mmol), after a
further 12h of stirring under N$_2$, the reaction mixture
was carefully neutralized with saturated NaHCO$_3$ solution
and then extracted with water (3 X 20ml). The combined
aqueous phase was back-extracted with CHCl$_3$ (30ml) and
the combined organic phase dried (MgSO$_4$) and evaporated
in vacuo to afford the diethyl thioacetal 22, which was
purified by flash chromatography (EtOAc/hexane, 1:1),

(2.21g, 87%). ^{1}H-NMR (CDCl$_3$): δ 1.06-1.34 (m, 6H), 2.15-2.75 (m, 6H), 3.51-3.60 (m, 1H), 3.68-3.81 (m, 1H), 4.45-4.60 (m, 2H), 4.70 and 4.32 (d, 1H, J=3.3Hz, rotamers), 5.01-5.38 (m, 2H), 7.27-7.40 (m, 5H). ^{13}C-NMR (CDCl$_3$): δ 14.5, 14.8, 26.4, 26.9, 36.1 (36.6), 52.9 (53.8), 56.0 (56.5), 60.4 (60.0), 66.7 (67.4), 69.9 (69.5), 127.7, 127.9, 128.3, 128.4, 128.5, 136.6 (136.0), 155.1. MS (m/z relative intensity): (EI) 355 (7), 337 (12), 308 (3), 220 (74), 176 (76), 135 (20), 91 (100), 65 (6). HRMS Calcd. for 355.1276 (C$_{17}$H$_{25}$O$_3$NS$_2$), found 355.1300.

(2S)-4(S)-Hydroxypyrrolidine-2-carboxaldehyde diethyl thioacetal (23)

A solution of Cbz-diethyl thioacetal (22;2.5g, 7.0mmol) in dry dichloromethane (15ml) was added dropwise over 5min to a stirred solution of trimethylsilyl iodide (2.8g, 14.0mmol) in CH$_2$Cl$_2$ (15ml) and the mixture stirred for 40min under N$_2$ atmosphere or until reaction was complete as indicated by TLC (EtOAc/hexane, 3:7). After quenching with methanol (15ml) and evaporation at 35°C in vacuo, the resulting residue was washed with hexane (2 x 100ml), purified by chromatography (CH$_3$OH/CHCl$_3$, 1:4) to afford 23 (1.25g, 80%) as a dark red low melting solid. ^{1}H-NMR (CDCl$_3$): δ 1.25-1.32 (m, 6H), 1.92-2.02 (m, 1H), 2.21-2.28 (m, 1H), 2.62-2.85 (m, 4H), 3.30-3.58 (m, 2H), 3.93-4.02 (m, 1H), 4.08 (d, 1H, J=8.0Hz), 4.55 (bs, 1H), 5.55 (bs, 2H). ^{13}C-NMR (CDCl$_3$): δ 14.4, 14.5, 24.5, 25.1, 39.2, 54.4, 54.5, 61.2, 70.9. IR (Neat): ν 3500-3060, 2960-2870, 1250-1150, 1020 cm^{-1}. MS (m/z, relative intensity) (CI, isobutane): 222 (100), 202 (3), 185 (3), 160 (37), 86 (11). HRMS Calcd. 221.0908 (C$_9$H$_{19}$ONS$_2$), found 221.0957.

1,1'-Propane-1,3-diyldioxybis(2-nitro-5-methoxy-1,4-phenylene)-bis(carbonyl)bis[4(S)-hydroxy-2(S)-pyrrolidine-2-carboxaldehyde diethylthio acetal] (24)

Dimethyl formamide (2 drops) was added to a stirred

suspension of the dimer acid (0.46g, 1.0mmol; see step
(iv) of Example 1) and oxalyl chloride (0.318g, 2.5mmol,
2.5 equiv.) in dry THF (20ml), and stirring continued
for 4 hours. After evaporation of the THF in vacuo, the
resultant yellow solid was dissolved in dry THF (10ml)
and added dropwise over a period of 30 minutes to a
vigorously stirred suspension of (2S)-4(S)-hydroxy-
pyrrolidine-2-carboxaldehyde diethyl thioacetal
(23;0.55g, 2.5mmol, 2.5 equiv.), Et$_3$N (0.42g. 4.2mmol,
4.2 equiv.) and ice/water (0.6ml) cooled in an ice bath.
After addition was complete, the mixture was warmed to
room temperature and stirred for a further 1.5 hours.
After removal of the THF by evaporation in vacuo, the
residue was diluted with water (2 x 40ml) and extracted
with ethyl acetate (4 X 25 ml). The aqueous phase was
adjusted to pH3 with conc. HCl and extracted with ethyl
acetate (2 X 25ml). The combined organic phase was
washed with water (3 X 25ml) and brine (3 X 25ml), dried
(anhyd. MgSO$_4$) and the solvent removed by evaporation in
vacuo to afford a dark red oil which was purified by
flash chromatography (ethyl acetate/hexane, 1:1; TLC:
ethyl acetate/hexane, 3:2) to afford the bis(amide) 24
as a pale yellow oil (0.64g, 73% yield). ^{1}H-NMR
(CDCl$_3$): δ 1.30-1.38 (m, 12H), 2.20-2.87 (m, 16H), 3.05-
3.16 (m, 2H), 3.42-3.48 (m, 2H), 3.95 (s, 6H), 4.30-4.45
(m, 6H), 4.83-4.89 (m, 4H), 6.82 (s, 2H), 7.64 (s, 2H).
^{13}C-NMR (CDCl$_3$): δ 14.9, 15.1, 26.3, 26.6, 28.9, 36.1,
52.4, 56.6, 58.3, 59.8, 65.1, 69.6, 108.0, 109,5, 127.8,
137.2, 148.2, 154.4, 167.0. IR (Neat):ν 3700-3160,
3040-2800, 1640, 1580, 1520, 1440, 1340, 1280, 1220,
1070, 970, 920, 870, 760 cm^{-1}. MS (FAB): m/z=837 M$^+$+1.

1,1'-Propane-1,3-diyldioxybis(2-amino-5-methoxy-1,4-
phenylene)-bis(carbonyl)bis[4(S)-hydroxy-2(S)-pyrroli-
dine-2-carboxaldehyde diethylthio acetal] 25
A solution of the dinitro thioacetal (24;0.5g, 0.57mmol)

and SnCl$_2$.2H$_2$O (1.34g, 6.0mmol) in methanol (25 ml) was
refluxed for 40 minutes until TLC (ethyl acetate/hexane,
4:1) indicated that the reaction was complete. The
solvent was removed by evaporation in vacuo and the
residue cooled to 0°C and treated with sat. NaHCO$_3$. The
resulting solid was then triturated with ethyl acetate
(3 X 50ml) and the mixture allowed to stir at room
temperature for 1 hour. The suspension was filtered
through a short bed of celite which was rinsed with
ethyl acetate (2 X 50ml). The combined filtrate was
evaporated in vacuo to afford the corresponding diamino
thioacetal as a light yellow foam. Further purification
by flash chromatography (ethyl acetate) afforded a
yellow oil 25 (0.35g, 75% yield), which was used
directly in the next step. ^{1}H-NMR (CDCl$_3$): δ 1.21-1.39
(m, 12H), 1.62-1.78 (bs, 2H), 2.01-2.18 (m, 2H), 2.21-
2.30 (m, 4H), 2.64-2.82 (m, 8H), 3.31-3.41 (m, 2H),
3.63-3.68 (m, 2H), 3.78 (s, 6H), 4.17-4.52 (m, 10H),
4.64-4.66 (m, 2H), 4.85-4.97 (m, 2H), 6.21 (s, 2H), 6.72
(s, 2H). IR (Neat): $\mathcal{V}$ 3710-3140, 3055-2820, 1650,
1595, 1520, 1470, 1460, 1450, 1420, 1340, 1270, 1240,
1180, 1120, 1070, 1030, 980, 870, 840, 760 cm^{-1}.

8,8'-[1,3-Propanediylbis(oxy)]bis[2(S),11(R,S),11a(S)-
7,11-dimethoxy-1,2,3,10,11,11a-hexahydro-2-hydroxy-5H-
pyrrolo[2,1-c][1,4]benzodiazepin-5-one] 26
A solution of the diamino thioacetal (25;0.4g, 0.5mmol),
HgCl$_2$ (0.81g, 3.0mmol, 6.0 equiv.), and CaCo$_3$ (0.3g,
3.0mmol, 6.0 equiv.) in CH$_3$CN/H$_2$O (4:1, 15ml) was
stirred slowly at room temperature for 2.5 hours until
TLC (MeOH/CHCl$_3$ 1:4) indicated a complete loss of
starting material. After removal of the CH$_3$CN/H$_2$O by
evaporation in vacuo at room temperature, the residue
was dissolved in MeOH (15ml) and loaded directly onto a
chromatography column. The column was first eluted with
ethyl acetate (HPLC grade, 5 X 100ml) to remove the

HgCl$_2$ salts, and then with CHCl$_3$ (HPLC grade, 2 X 100ml). Gradient elution with MeOH/CHCl$_3$ (6-10% MeOH) afforded 26 (0.18g, 57%) as a light yellow oil. ^{1}H-NMR (CD$_3$OD) mixture of C11(R,S)-OCH$_3$ isomers): δ 2.21-2.46 (m, -CH$_2$-CH$_2$-CH$_2$- and 4 X H1), 2.92-3.18 (m, 2 X H3), 3.60-3.98 (m, 2 X C7-OCH$_3$, 2 X H11a), 4.12-4.39 (m, O-CH$_2$-CH$_2$-CH$_2$-O-, 2 X H2, 2X C$_2$-OH), 4.41-4.58 (m, 2X H11), 6.39 and 7.38 (s, major; 4H), 7.08 and 7.57 (s, minor, 4H). Small amount of imine was observed as doublet at δ 8.20 for C11 and singlets at δ 6.60 and 7.18 for aromatic protons. ^{13}C-NMR (CDCl$_3$) (mixture of C11 (R,S) diastereomers and small amount of imine): δ 30.1, 39.6, 58.2, 66.2 (-CH$_2$-, weaker signals for diastereomers observed at 42.7, 55.0, 56.4, 66.7), 57.2, 59.7, 66.5, 69.4 (CH$_3$O- and -CH-, additional signal for diastereomer observed at 56.6), 88.6 (C11), 102.9, 106.3, 108.7, 116.3 (C6 and C9), 141.7, 142.9, 146.3, 150.5, 154.3, 156.1, 159.2, 162.1 (C5a, C7, C8, C9a), 169.1 (C=O) other weak signals respectively the imine species were observed at, for example, 110.0, 113.9, 140.0, 146.0, 153.4, 168.9, 171.1 (C11) for the aromatics. MS (FAB): m/z = 565 (M$^+$-2 X CH$_3$OH).

Example 9

Experiments have been carried out to determine the concentration of PBD dimers according to the invention required to obtain 50% cross-linking of pBR332 DNA under standard conditions.

In vitro cytotoxicity studies have also been carried out. Compounds were evaluated in the following three cell lines: the L1210 mouse leukaemia (antimetabolite sensitive), the ADJ/PC6 mouse plasmacytoma (alkylating agent sensitive), and the human ovarian CH1 cell line (platinum sensitive). L1210 and ADJ/PC6 were grown as suspension cultures, whereas CH1 was grown as a monolayer. Experimental protocols were as follows:

1210 Leukaemia

Day 0   Cells were set up at 10^4mL in RPMI 1640 medium
         containing 10% horse serum.

Day 1   Cells were counted to ensure that they were in
         the logarithmic phase of growth, and then treated
         with the test compound which was left in contact
         with the cells for 48h at 37°C.

Day 3   Cells were counted and the percentage inhibition
         of growth calculated by comparison with controls,
         which were treated with solvent alone.

ADJ/PC6 Plasmacytoma

Day 0   Cells were set up at 3-5 X 10^4/mL in Dulbecco's
         medium containing 20% horse serum.

Day I   Cells were counted to ensure they were in the
         logarithmic phase of growth, and then treated
         with the test compound which was left in contact
         with the cells for 72H at 37°C.

Day 4   Cells were counted and the percentage inhibition
         of growth calculated by comparison with controls,
         which were treated with solvent alone.

CH1 Human Ovarian

Day 0   Cells were set up in flasks (100-200 cells/
         flask), in Dulbecco's medium containing 10%
         foetal calf serum, and left overnight to allow
         the cells to attach.

Day 1   The test compound was added and left in contact
         with the cells for the duration of the
         experiment, at 37°C.

Day 9   Colonies of cells were usually visible and were
         fixed in ethanol (100%), stained with methylene
         blue and counted.

         Compounds were screened over a concentration
range starting at 10^{-4}M and decreasing factors of ten
until 10^{-11}M. Each compound was dissolved in DMSO so

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that the final concentration of DMSO was 0.05% in
contact with the cells. Suspension cultures were
counted using a Coulter counter and the CH1 colonies
counted with a colony counter. The index of activity is
expressed as the IC_{50}, that is the dose which inhibits
cell growth by 50% compared to the solvent controls.

The PBD dimers were also tested for activity
against K_{562} human cell line; IC_{50} values were measured
using a MTT assay following one hour exposure to test
ligand.

The table below summarises the results (for
DSB-120 this table repeats certain results previously
given):

| Compound	50% cross-linking pBR322, µM	IC_{50} µM			
		L1210	ADJ/PC6	CH1	K_{562}
DSB-120	0.055	0.01	0.0005	0.003	0.2
AT 111 (Ex.3)	1.00	1.2	0.35	0.05	2.5
AT-286 (Ex.4)	0.07	0.0045	0.0004	0.00032	0.5
DSB-131 (Ex.5)	0.75	0.34	0.002	0.002	1.0
Ex. 6	0.01	0.00028	0.00008	–	0.01
Ex. 7	0.02	0.22	0.036	–	3.5

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CLAIMS:

1.   Pyrrolo [2,1-c][1,4] benzodiazepine (PBD) dimers of the formula:

where the PBD tricyclic structures may contain additional substituents in one or more of the 1, 2, 3, 6, 7, 9 and 11- positions and the C rings may optionally contain at least one further hetero ring atom, X is -O-, -S- or -NH-, and R^1 is an alkylene chain containing from 3 to 12 carbon atoms, which chain may, if desired, be interrupted by one or more hetero-atoms and which chain may, if desired, contain one or more carbon-carbon double or triple bonds.

2.   PBD dimers according to claim 1, in which the tricyclic structures each contain one or more hydroxyl, alkoxy, ethylidene or 2-acrylamidyl substituents.

3.   PBD dimers according to claim 1 or 2, which are substituted in the 2- and 2'- positions by hydroxyl groups or substituents of the formula:

$$-O(CH_2)_n-NH_3^+Cl^-$$

where n is an integer from 1 to 6.

4.   PBD dimers according to any of claims 1 to 3,
in which the 10,11- positions of the tricyclic
structures are in the carbinolamine form:

$$- NH - C^{OH} -$$
$$\quad 10 \quad 11$$

or a derivative thereof.

5.   PBD dimers according to claim 4, in which the
11-position is substituted by an alkoxy or thioalkyl
group.

6.   PBD dimers according to claim 4, in which the
11- position is substituted by the residue of a
nucleophilic reagent.

7.   PBD dimers according to claim 6, in which the
nucleophilic reagent is ethylamine, thiophenol, or
cysteine.

8.   PBD dimers according to any of claim 1 to 7,
in which R^1 is $-(CH_2)_x-O-(CH_2)_x-$, where x is an integer
from 2 to 6, or $-(CH_2)_n-CH=CH-(CH_2)_n-$, where n is an
integer from 1 to 5.

9.   PBD dimers according to any of claims 1 to 8,
in which the C rings contain an -O- ring atom in the 1-
and 1'- positions.

10.   The PBD dimer according to claim 1, in which
there are methoxy substituents in the 7- and 7'-
positions, X is 0 and R^1 is propylene.

11.   A method of preparing a PBD dimer of formula I specified in claim 1, which comprises:

        (i)   condensing a benzoic acid derivative of the formula:

II

where X has the meaning specified in claim 1, R^2 is H or a desired substituent in the 6- or 6'- position of the PBD dimer, R^3 is H or a desired substituent in the 7- or 7'- position of the PBD dimer, and R^4 is H or a desired substituent in the 9- or 9'- position of the PBD dimer, with a dihaloalkane derivative of the formula:

        $$X-R^1-X$$ III

where R^1 has the meaning specified in claim 1 and X is a halogen atom, by adding the dihaloalkane III slowly to a reaction mixture containing the benzoic acid derivative II to form a dimer acid compound of the formula:


IV

(ii) esterifying the dimer acid of formula IV,

(iii) nitrating the diester from step (ii) to obtain a dinitro-diester compound of the formula:

$$O_2N-\underset{R^2}{\overset{R^4}{\bigodot}}-X-R^1-X-\underset{R^2}{\overset{R^4}{\bigodot}}-NO_2 \qquad V$$

R^{5}OOC ... R^3 ... R^3 ... COOR^5

where R^5 is an alkyl group,

(iv) hydrolysing the dinitro-diester compound of formula V to remove the esterifying groups and obtain the corresponding dinitro-diacid compound,

(v) condensing the dinitro-diacid compound from step (iv) with a (2S)-pyrrolidine-2-carbaldehyde diethyl thioacetal of the formula:

$$\underset{HN}{\overset{CH(SC_2H_5)_2}{\bigodot}}\begin{matrix}R^6\\R^7\\R^8\end{matrix} \qquad VI$$
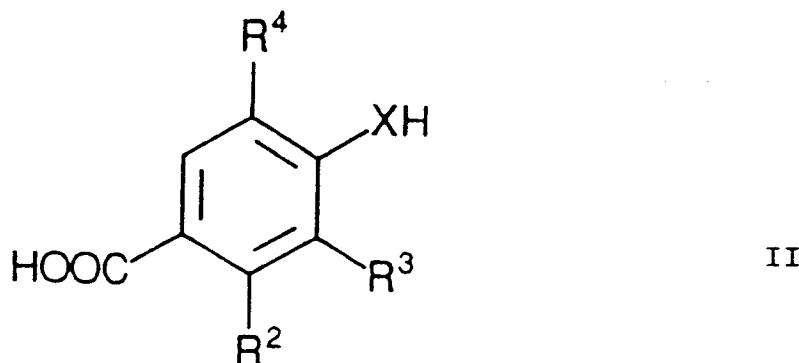
where R^6 is H or a desired substituent in the 1- or 1'-position of the PBD dimer, R^7 is H or a desired substituent in the 2- or 2'- position of the PBD dimer,

and R^8 is H or a desired substituent in the 3- or 3'-position of the PBD dimer, to form a nitrothioacetal derivative of the formula:

(vi)   reducing the nitro substituents of the derivative of formula VII to amino groups, and

(vii) subjecting the diamino compound from step (vi) to ring closing conditions whereby a PBD dimer of formula I is obtained.

9.   A method according to claim 8, in which step (vii) is carried out so as to leave an $-SC_2H_5$ substituent in the 11- and 11'- positions of the PBD dimer obtained (the nitrogen in the 10- and 10'-positions being in the amine form).

10.   A method according to claim 8 or 9, in which the condensation reaction of step (i) is carried out by slowly adding a solution of the dihaloalkane III in an organic solvent to a two-phase reaction medium containing the benzoic acid derivative II, the reaction medium comprising an organic solvent which is the same as or miscible with the organic solvent present in the dihaloalkane III solution and an aqueous alkali.

11.   A pharmaceutical composition comprising a PBD

dimer of formula I specified in claim 1 and an inert, physiologically acceptable carrier.

12.   A pharmaceutical composition according to claim 1, in the form of an injectable solution, a formulation for oral administration, or a composition for topical application.

13.   A method of treating cancer in a human or animal patient, which comprises the step of administering to said patient an effective amount of a pyrrolobenzodiazepine derivative as set forth in claim 1.

INTERNATIONAL SEARCH REPORT

International Application No   PCT/GB 93/00483

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D519/00;   A61K31/55;   //(C07D519/00,487:00,487:00)
(C07D519/00,498/00,498/00)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; A61K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[o]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	TETRAHEDRON LETTERS vol. 29, no. 40, 1988, OXFORD GB pages 5105 - 5108 J.W. SUGGS ET AL. 'Synthesis and DNA crosslinking ability of a dimeric anthramycin analog' cited in the application see complete article	1,14
P,X	JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS no. 20, 15 October 1992, LETCHWORTH GB pages 1518 - 1520 D.E. THURSTON ET AL. 'Effect of linker length on DNA-binding affinity, cross-linking efficiency and cytotoxicity of c8-linked pyrrolobenzodiazepine dimers' see complete article	1,11,14

° Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23 JUNE 1993	30. 06. 93

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	ALFARO FAUS I.

Form PCT/ISA/210 (second sheet) (January 1985)

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 16 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/compositon."

Remark: Claims 9 to 13 on pages 45 and 46 have been renumbered 12 to 16

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.